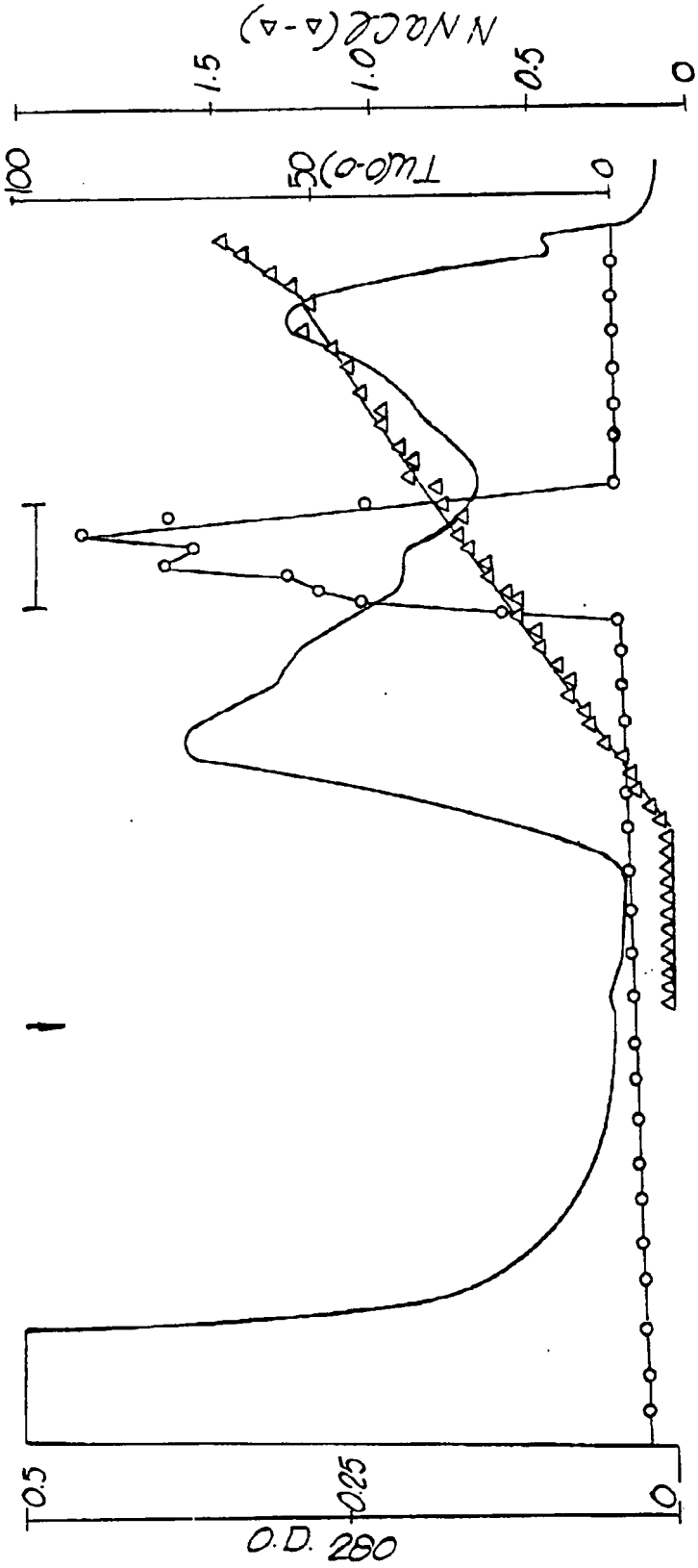

FIG.1

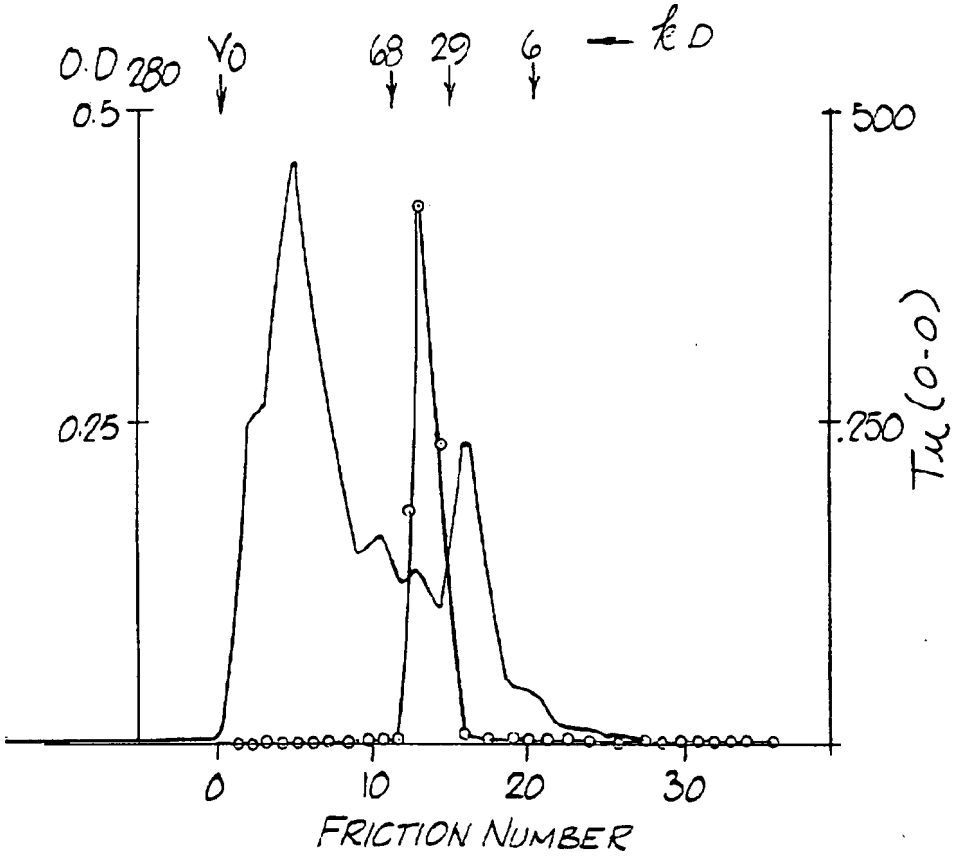
O.D 280
Vo
68
29
6
kD
0.5
0.25
500
250
Tu (O-O)
0
10
20
30
FRICTION NUMBER

FIG. 2

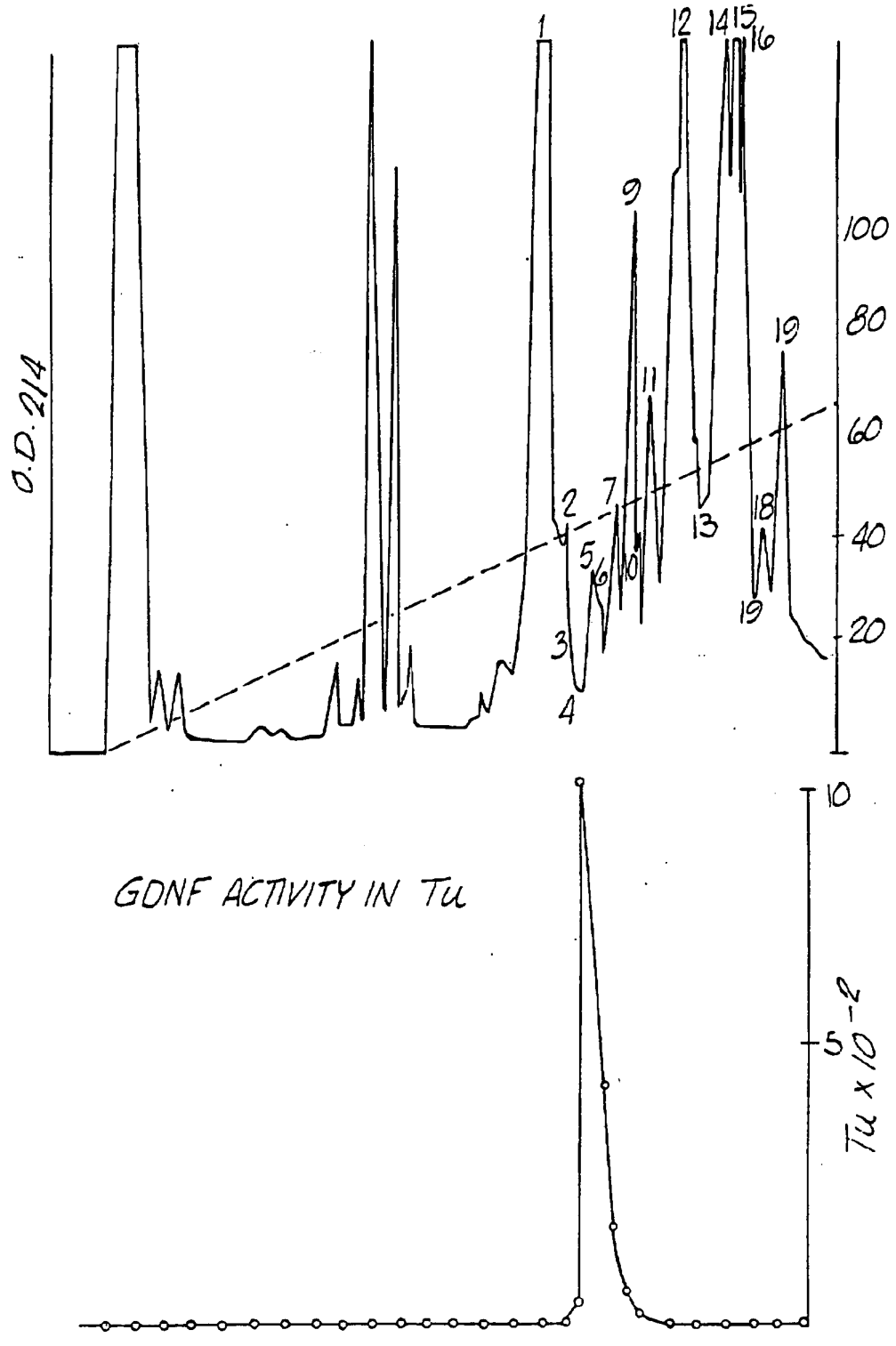

FIG. 3

SUBSTITUTE SHEET

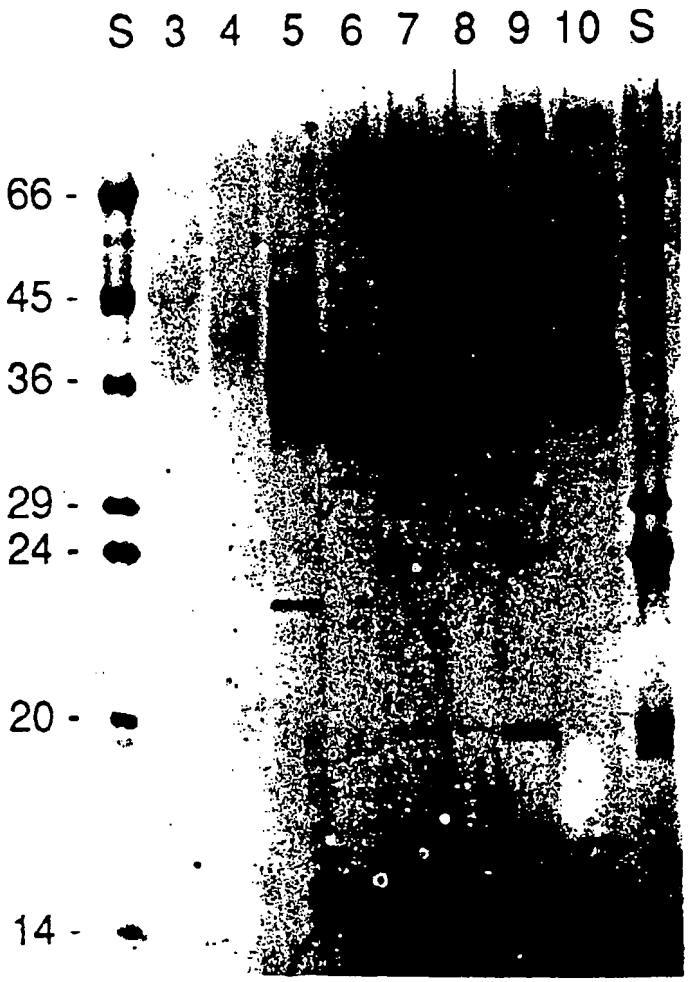

FIG. 4

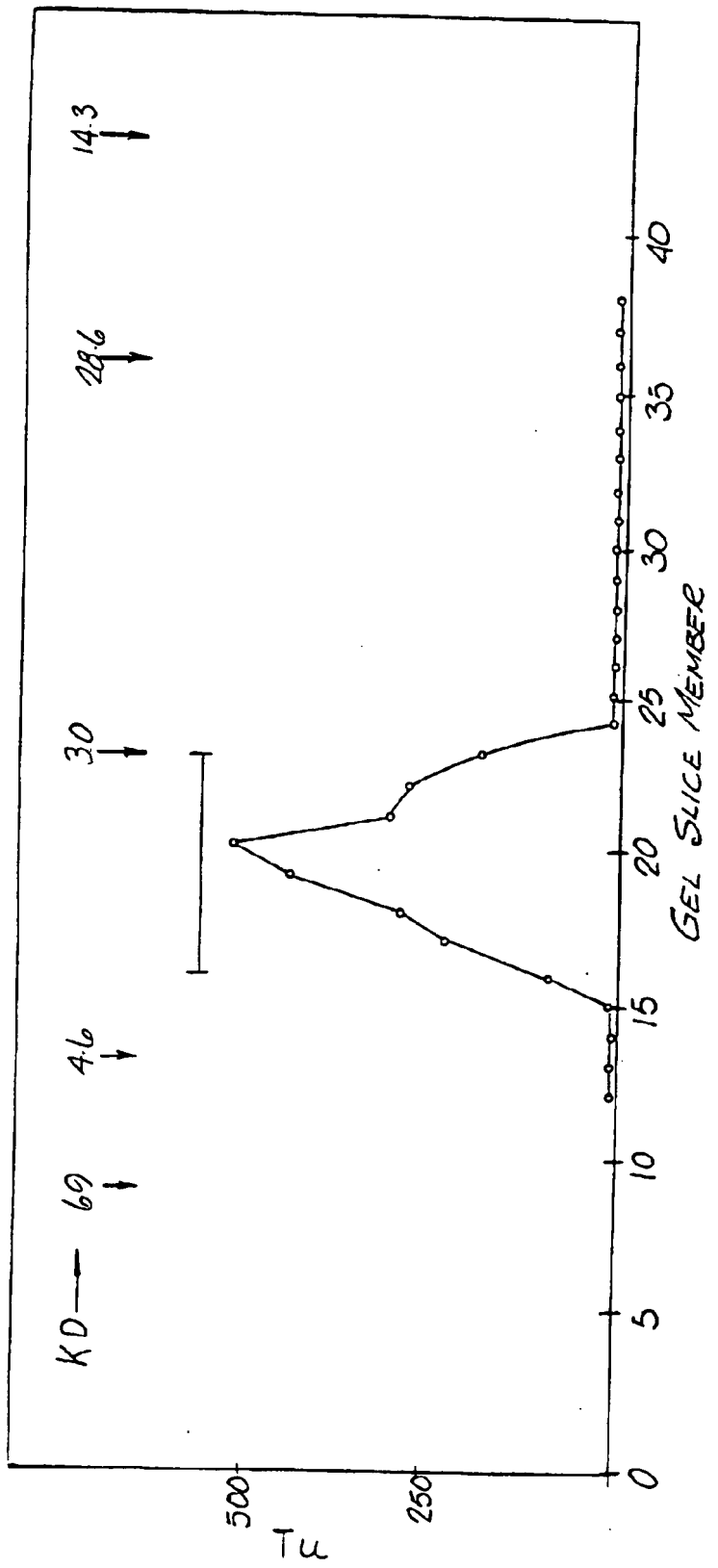
14.3
28.6
30
4.6
69
KD
500
250
Tu
0
5
10
15
20
25
30
35
40
GEL SLICE MEMBER

FIG.5

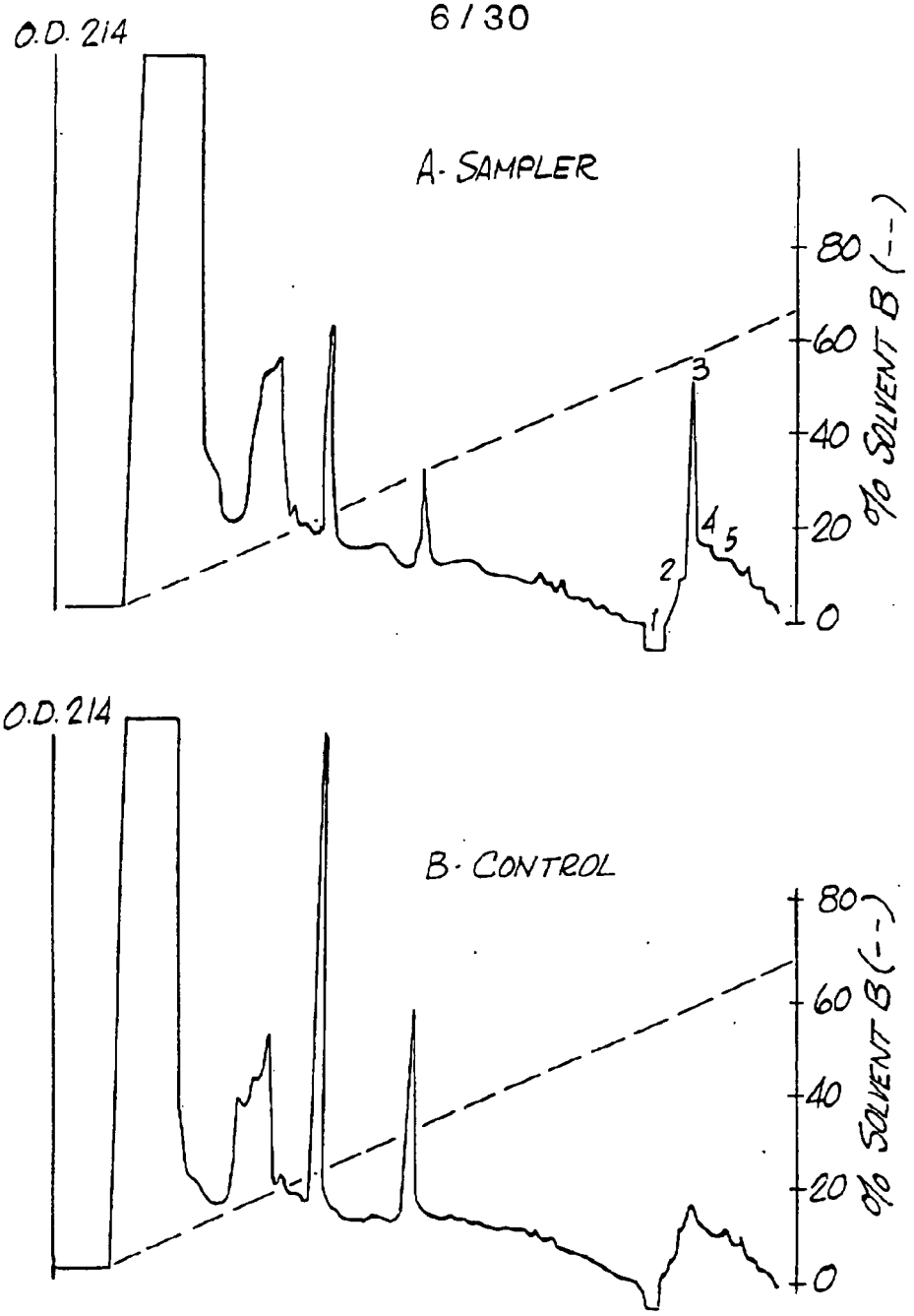
O.D. 214
A. SAMPLER
80
60
40
20
0
% SOLVENT B (--)
1
2
3
4
5
O.D. 214
B. CONTROL
80
60
40
20
0
% SOLVENT B (--)

FIG. 6

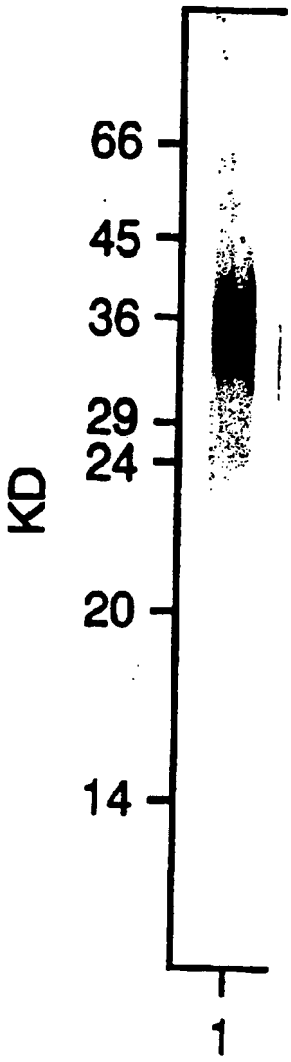

FIG. 7

FIGURE 8

Amino-terminal sequence of GDNF

(Ser)-Pro-Asp-Lys-Gln-Ala-Ala-Ala-Leu-Pro-Arg-Arg-Glu-
(Arg)-Asn-( )*-Gln-Ala-Ala-Ala-Ala-(Ser)-Pro-(Asp)-(Asn)

* no residue could be unequivocally identified in this position

amino acid residues in parenthesis are those identified with less certainty

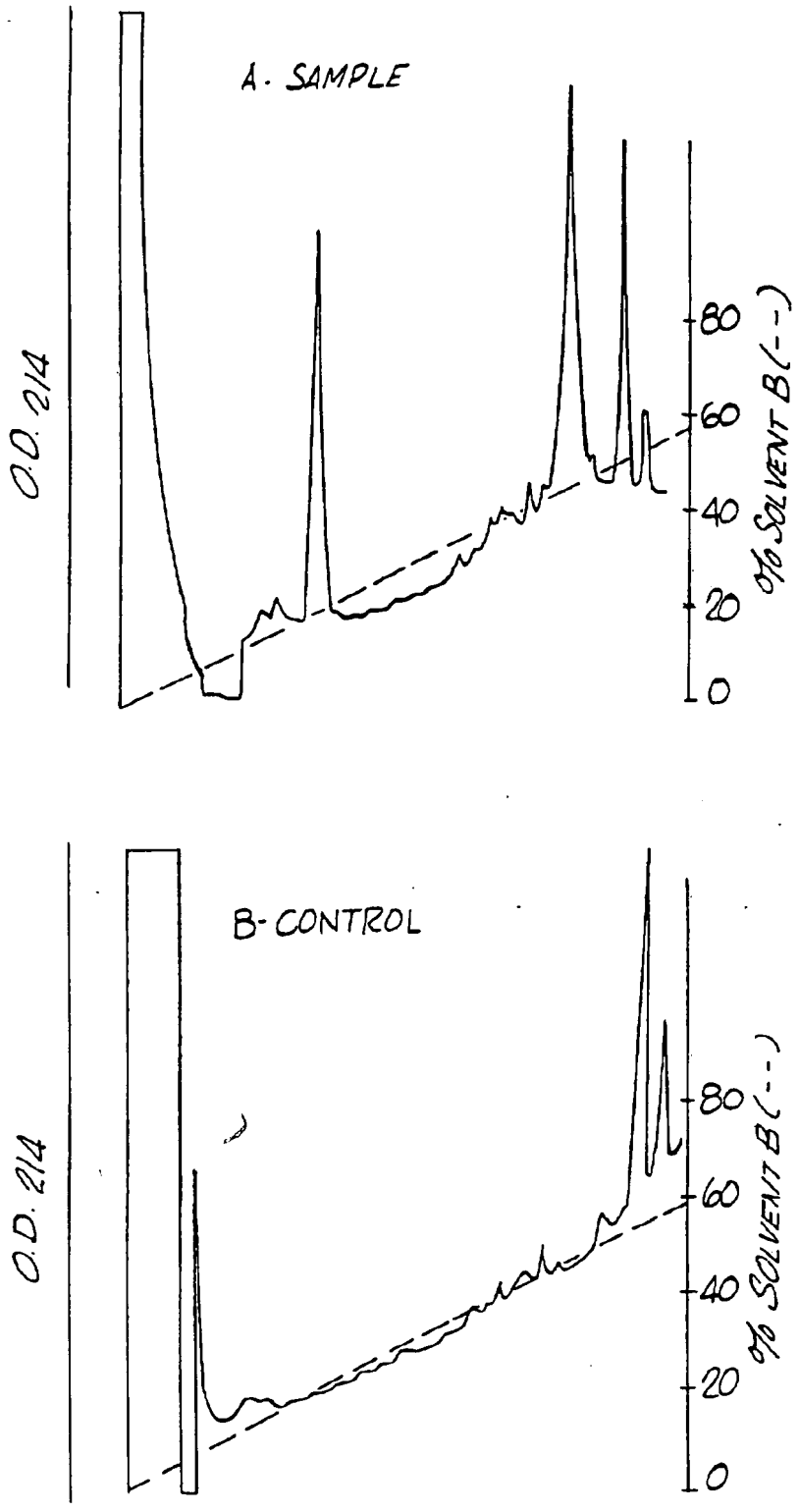
A. SAMPLE
O.D. 214
80
60
40
20
0
% SOLVENT B(- -)
B- CONTROL
O.D. 214
80
60
40
20
0
% SOLVENT B(- -)

FIG.9

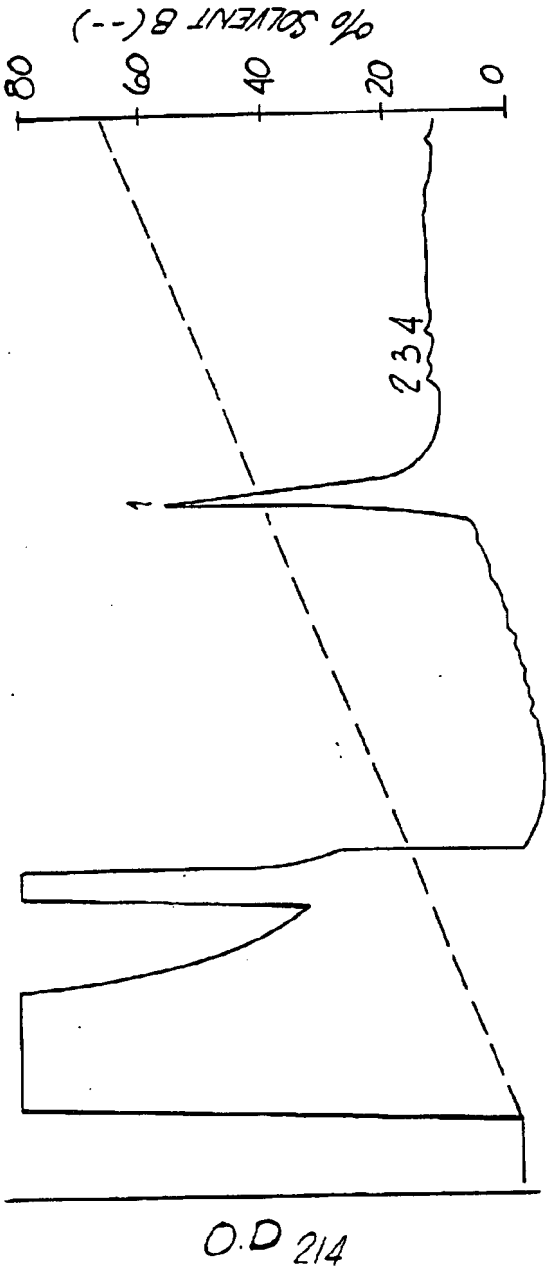

FIG.10

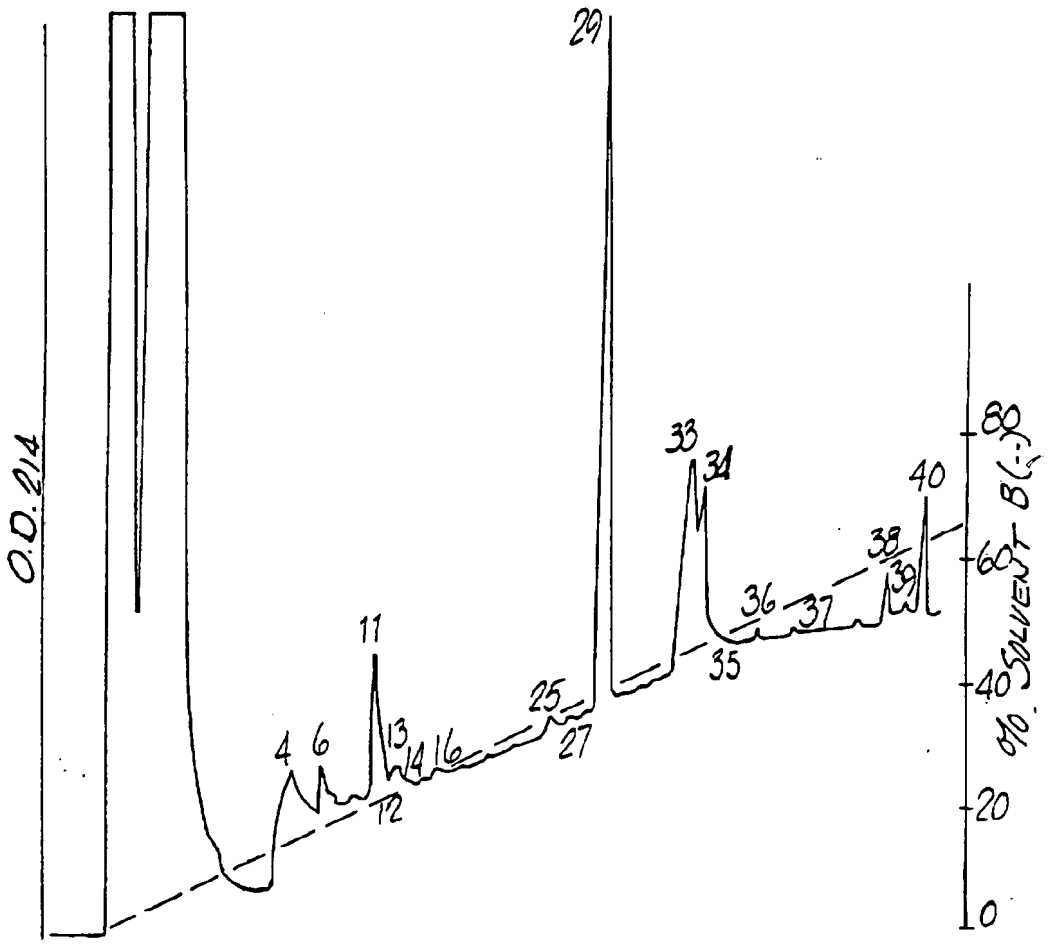
O.D. 214
29
33
34
40
38
39
36
37
11
4
6
13
14
16
25
27
12
35
80
60
40
20
0
% SOLVENT B(--)

FIG.11

FIGURE 12

An internal sequence of the GDNF

Asp-(Lys/Gln)-Ile-Leu-Lys-Asn-Leu-(Gly)*-(Arg)-(Val)-(Arg)-(Arg)-Leu

*Amino acid residues placed in parentheses are ones identified with less certainty.

```
                                        1                                                                   34
CCCCCGGGCT GCAGGAATTC GGGG GTC TAC GGA GAC CGG ATC CGA GGT GCC GCC GCC
                           V   Y   G   D   R   I   R   G   A   A   A

                                                                                                        88
GGA CGG GAC TCT AAG ATG AAG TTA TGG GAT GTC GTG GCT GTC TGC CTG GTG TTG
G   R   D   S   K   M   K   L   W   D   V   V   A   V   C   L   V   L
                    *

                                                                                                       142
CTC CAC ACC GCG TCT GCC TTC CCG CTG CCC GCC GGT AAG AGG CTT CTC GAA GCG
L   H   T   A   S   A   F   P   L   P   A   G   K   R   L   L   E   A

                                                                                                       196
CCC GCC GAA GAC CAC TCC CTC GGC CAC CGC CGC GTG CCC TTC GCG CTG ACC AGT
P   A   E   D   H   S   L   G   H   R   R   V   P   F   A   L   T   S

                                                                                                       250
GAC TCC AAT ATG CCC GAA GAT TAT CCT GAC CAG TTT GAT GAC GTC ATG GAT TTT
D   S   N   M   P   E   D   Y   P   D   Q   F   D   D   V   M   D   F

                                                                                                       304
ATT CAA GCC ACC ATC AAA AGA CTG AAA AGG TCA CCA GAT AAA CAA GCG GCG GCA
I   Q   A   T   I   K   R   L   K   R   S   P   D   K   Q   A   A   A

                                                                                                       358
CTT CCT CGA AGA GAG AGG AAC CGG CAA GCT GCA GCT GCC AGC CCA GAG AAT TCC
L   P   R   R   E   R   N   R   Q   A   A   A   A   S   P   E   N   S
```

FIG. 13

412
AGA GGG AAA GGT CGC AGA GGC CAG AGG GGC AAA AAT CGG GGG TGC GTC TTA ACT
R G K G R R G Q R G K N R G C V L T

466
GCA ATA CAC TTA AAT GTC ACT GAC TTG GGT TTG GGC TAC GAA ACC AAG GAG GAA
A I H L N V T D L G L G Y E T K E E

520
CTG ATC TTT CGA TAT TGT AGC GGT TCC TGT GAA GCG GCC GAG ACA ATG TAC GAC
L I F R Y C S G S C E A A E T M Y D

574
AAA ATA CTA AAA AAT CTG TCT CGA AGT AGA AGG CTA ACA AGT GAC AAG GTA GGC
K I L K N L S R S R R L T S D K V G

628
CAG GCA TGT TGC AGG CCG GTC GCC TTC GAC GAC GAC CTG TCG TTT TTA GAC GAC
Q A C C R P V A F D D D L S F L D D

682
AGC CTG GTT TAC CAT ATC CTA AGA AAG CAT TCC GCT AAA CGG TGT GGA TGT ATC
S L V Y H I L R K H S A K R C G C I

745
—>
TGA CCCTGGCTCC AGAGACTGCT GTGTATTGCA TTCCTGCTAC AGTGCGAAGA AAGGGACCAA

815
GGTTCCCAGG AAATATTTGC CCAGAAAGGA AGATAAGGAC CAAGAAGGCA GAGGCAGAGG CGGAAGAAGA

875
AGAAGAAAAG AAGGACGAAG GCAGCCATCT GTGGGAGCCT GTAGAAGGAG GCCCAGCTAC AG

FIG 13 (CONT)

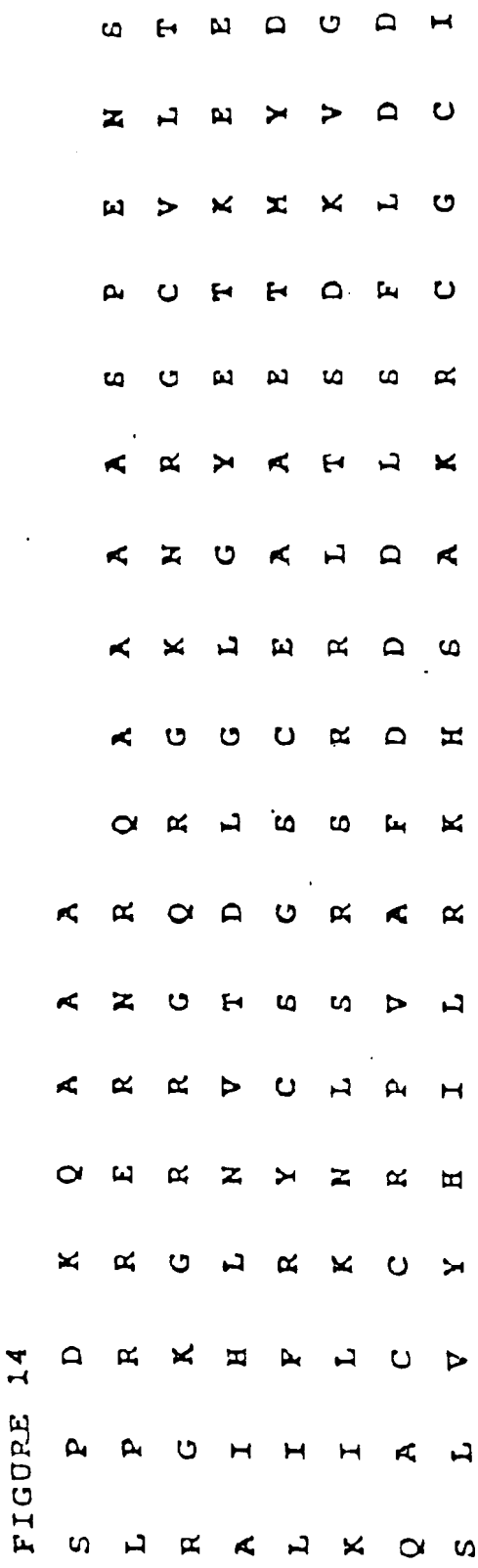
S L R A L K Q S
P P G I I I A L
D R K H P L C V
K R G L R K C Y
Q E R N Y N R H
A R R V C L P I
A A N G T S V L
A R Q D G R A R
Q R L S S F K
A G G C R D H
A K L E R D S
A N G A L D A
A R Y A T L K
S G E E S S R
P C T T D F C
E V K M K L G
N L E Y V D C
S T E D G D I

FIGURE 14

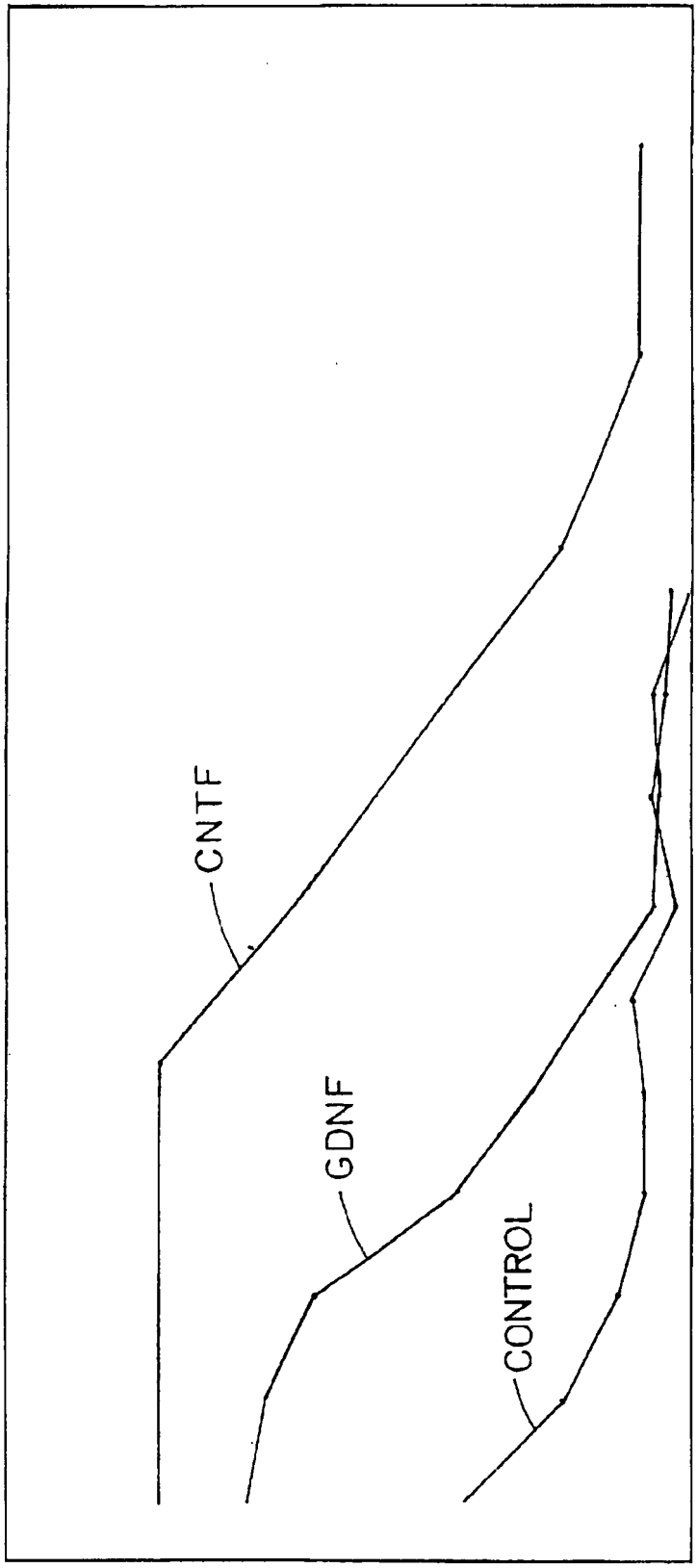

FIG.15

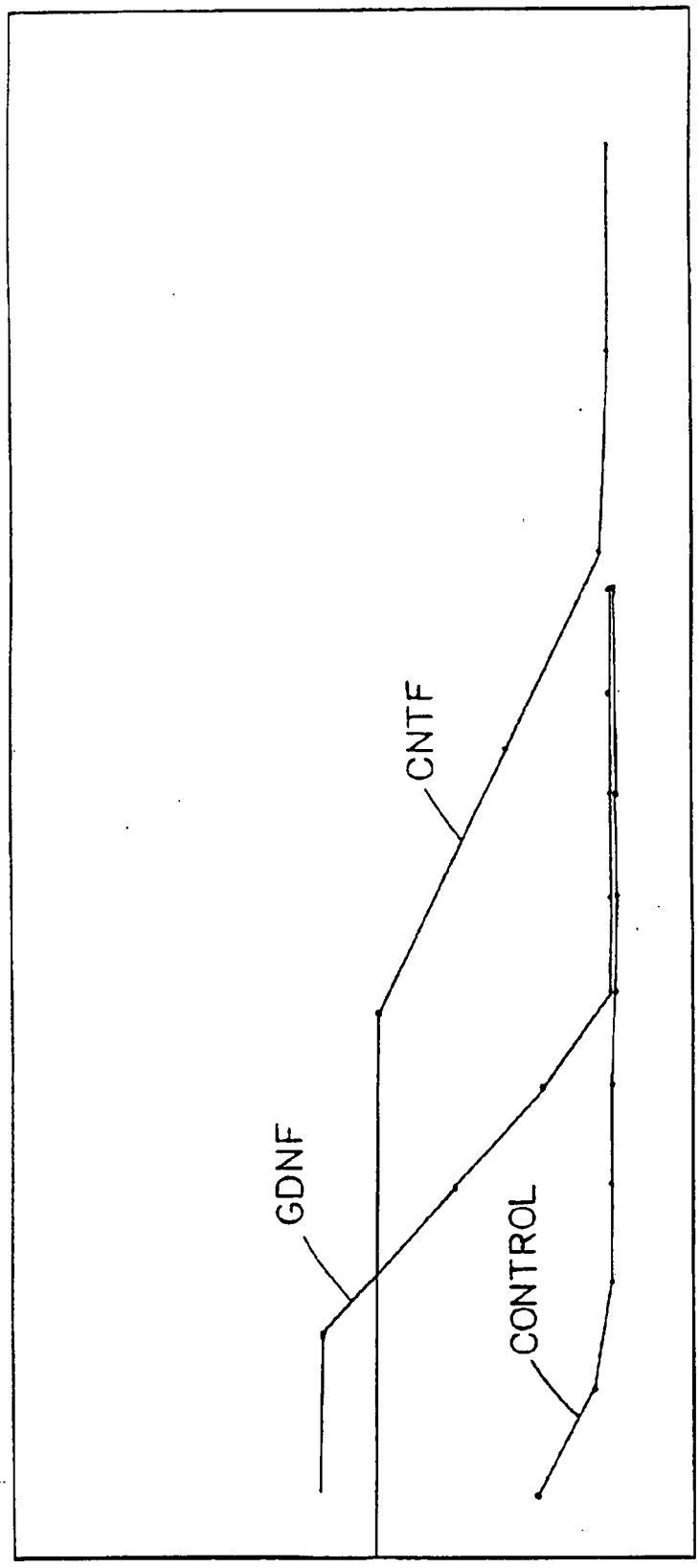

FIG.16

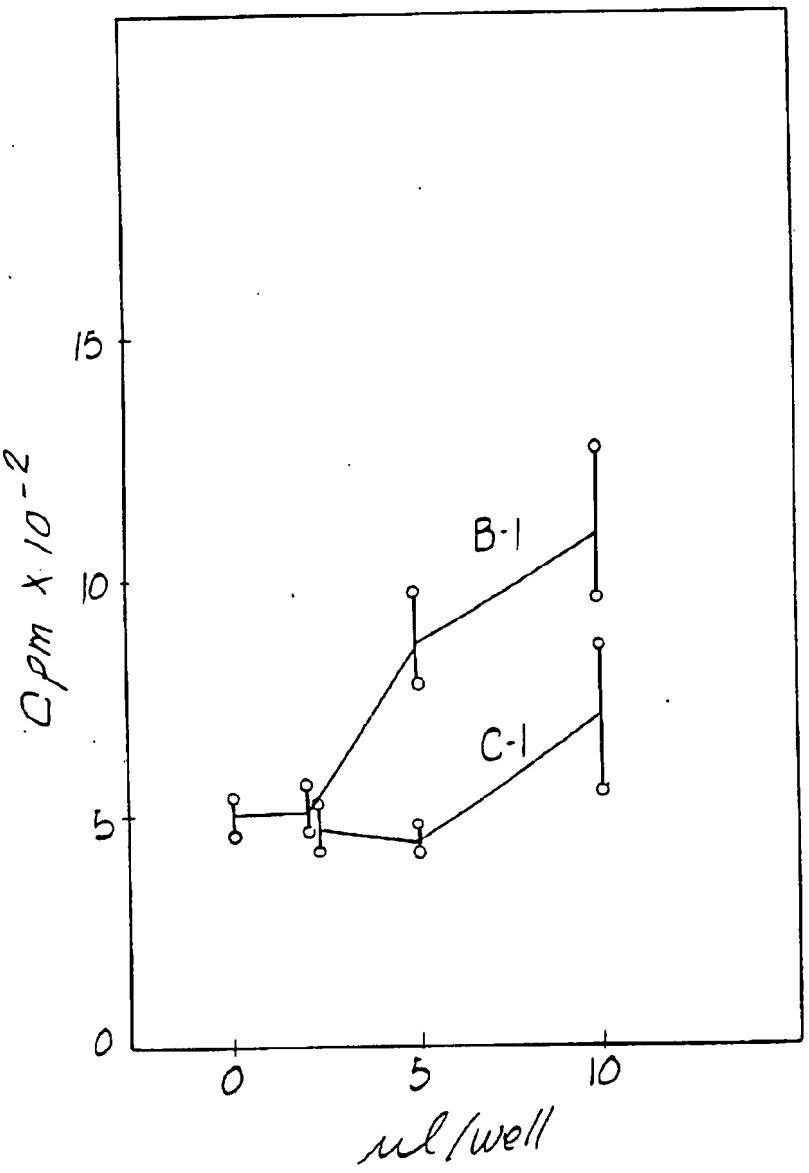

FIG. 17

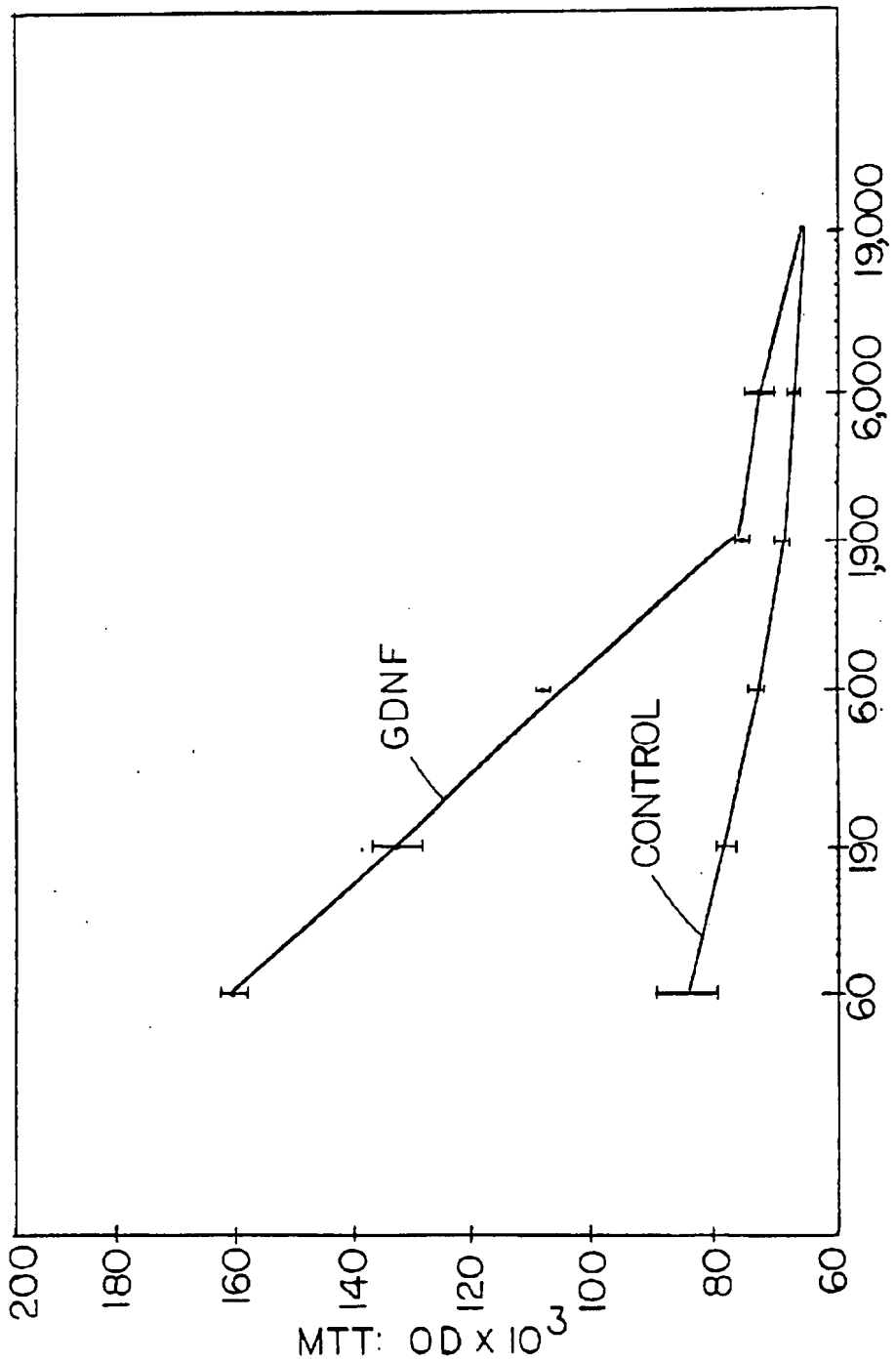
GDNF
CONTROL
200
180
160
140
120
100
80
60
MTT: OD X 10^3
60
190
600
1,900
6,000
19,000
DILUTION IN ASSAY

FIG.18


attttctctttctttttgaacag >CA AAT ATG CCA GAG GAT TAT CCT GAT CAG TTC GAT GAT GTC ATG 68
N M P E D Y P D Q F D D V M
GAT TTT ATT CAA GCC ACC ATT AAA AGA CTG AAA AGG TCA CCA GAT AAA CAA ATG GCA GTG CTT 131
D F I Q A T I K R L K R S P D K Q M A V L
CCT AGA AGA GAG CGG AAT CGG CAG GCT GCA GCT GCC AAC CCA GAG AAT TCC AGA GGA AAA GGT 194
P R R E R N R Q A A A A N P E N S R G K G
CGG AGA GGC CAG AGG GGC AAA AAC CGG GGT TGT GTC TTA ACT GCA ATA CAT TTA AAT GTC ACT 257
R R G Q R G K N R G C V L T A I H L N V T
GAC TTG GGT CTG GGC TAT GAA ACC AAG GAG GAA CTG ATT TTT AGG TAC TGC AGC GGC TCT TGC 320
D L G L G Y E T K E E L I F R Y C S G S C
GAT GCA GCT GAG ACA ACG TAC GAC AAA ATA TTG AAA AAC TTA TCC AGA AAT AGA AGG CTG GTG 383
D A A E T T Y D K I L K N L S R N R R L V
AGT GAC AAA GTA GGG CAG GCA TGT TGC AGA CCC ATC GCC TTT GAT GAT GAC CTG TCG TTT TTA 446
S D K V G Q A C C R P I A F D D D L S F L

FIG.19

GAT GAT AAC CTG GTT TAC CAT ATT CTA AGA AAG CAT TCC GCT AAA AGG TGT GGA TGT ATC TGA 509
D D N L V Y H I L R K H S A K R C G C I . 562

ctccggctccagagactgctgtgtattgcattcctgctacagtgcaaagaaag

FIG.19 (CON'T)

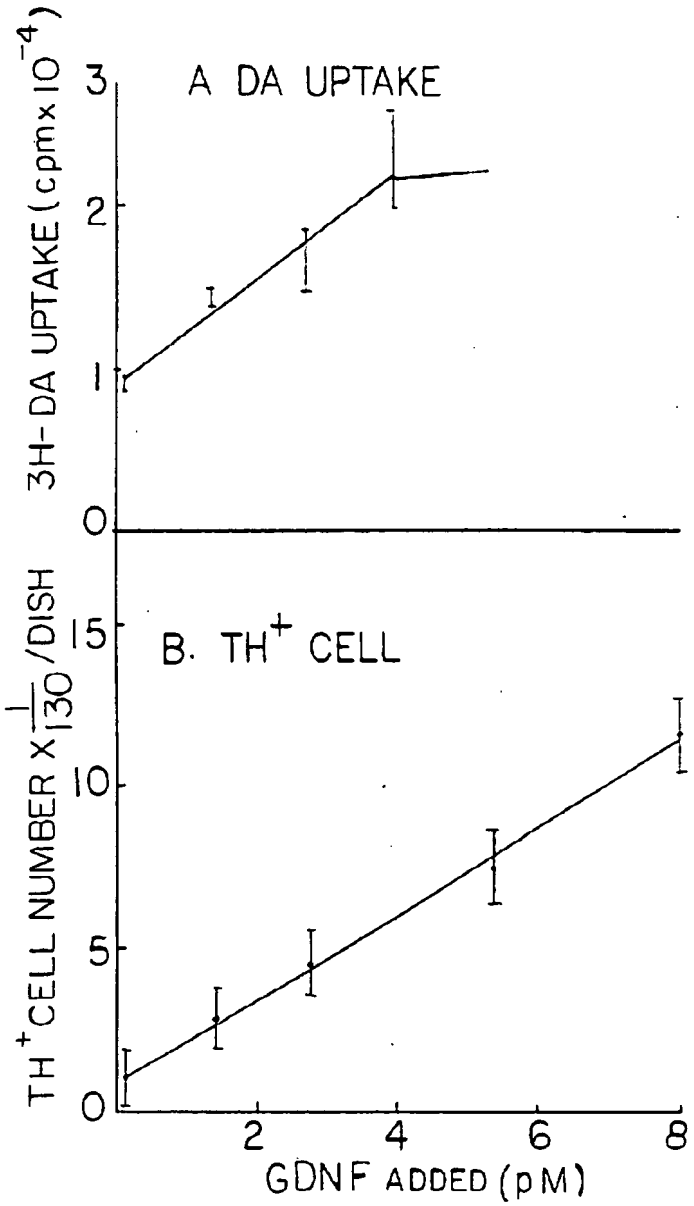
A DA UPTAKE
3H- DA UPTAKE (cpm x 10^{-4})
3
2
1
0
B. TH$^+$ CELL
TH$^+$ CELL NUMBER X $\frac{1}{130}$ /DISH
15
10
5
0
2
4
6
8
GDNF ADDED (pM)

FIG. 20

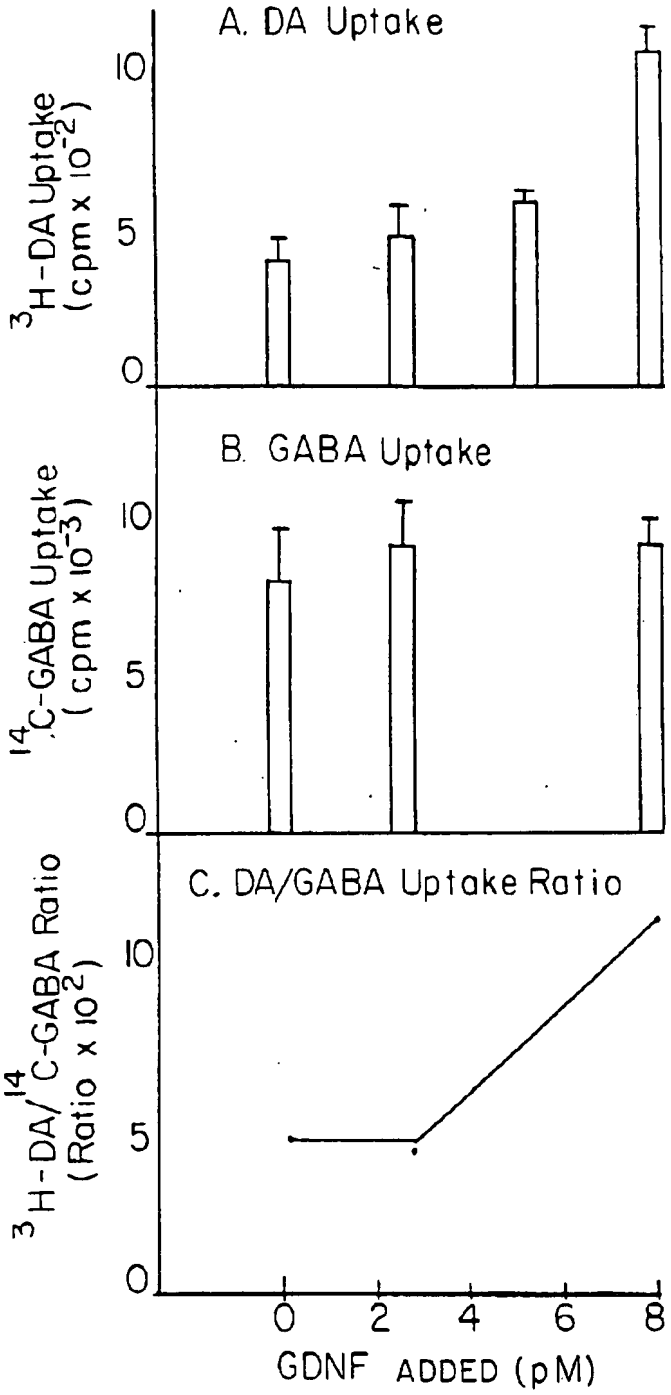
A. DA Uptake
^{3}H-DA Uptake (cpm x 10^{-2})
10
5
0
B. GABA Uptake
^{14}C-GABA Uptake (cpm x 10^{-3})
10
5
0
C. DA/GABA Uptake Ratio
^{3}H-DA/^{14}C-GABA Ratio (Ratio x 10^2)
10
5
0
0
2
4
6
8
GDNF ADDED (pM)

FIG.21

4
ttctctccccacctcccgcctgcccgcgca gGT GCC GCC GCC
G A A A

97
GGA CGG GAC TTT AAG ATG AAG TTA TGG GAT GTC GTG GCT GTC TGC CTG GTG CTG
G R D F K M K L W D V V A V C L V L
*

151
CTC CAC ACC GCG TCC GCC TTC CCG CTG CCC GCC GGT AAG AGG CCT CCC GAG GCG
L H T A S A F P L P A G K R P P E A

205
CCC GCC GAA GAC CGC TCC CTC GGC CGC CGC CGC GCG CCC TTC GCG CTG AGC AGT
P A E D R S L G R R R A P F A L S S

223
GAC Tgtaagaaccgttcc
D

FIG.22

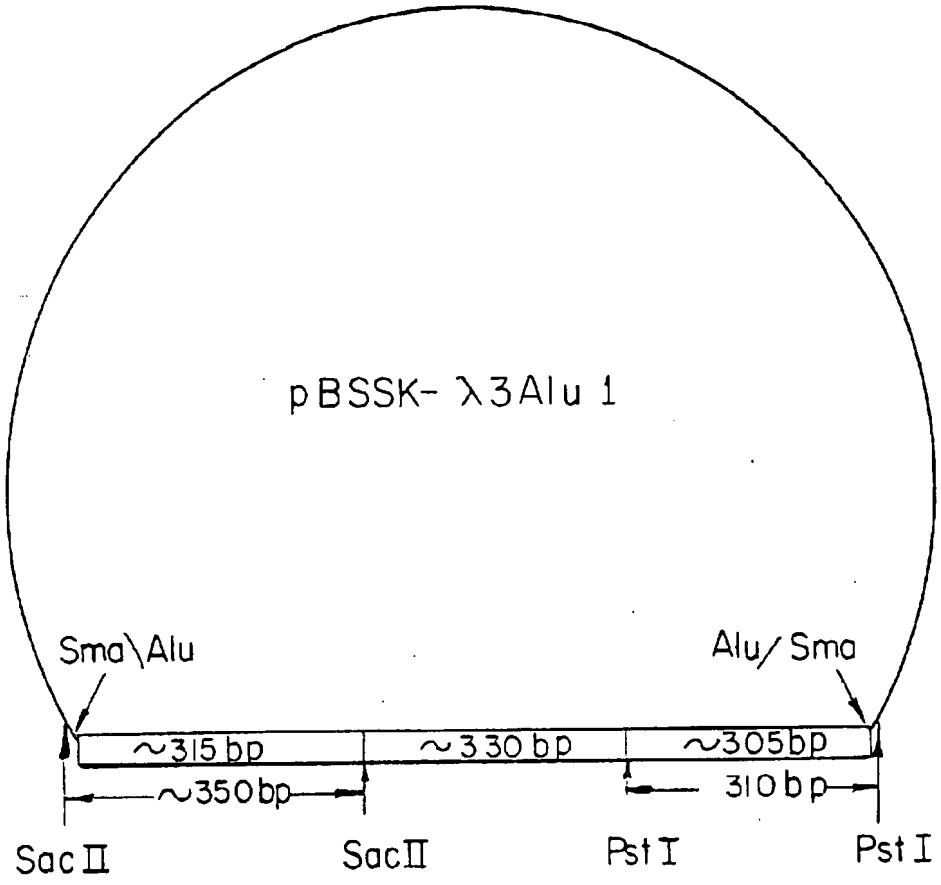
pBSSK- λ3Alu 1
Sma\Alu
Alu/ Sma
~315 bp
~330 bp
~305bp
~350bp
310 bp
Sac II
SacII
Pst I
Pst I

FIG. 23

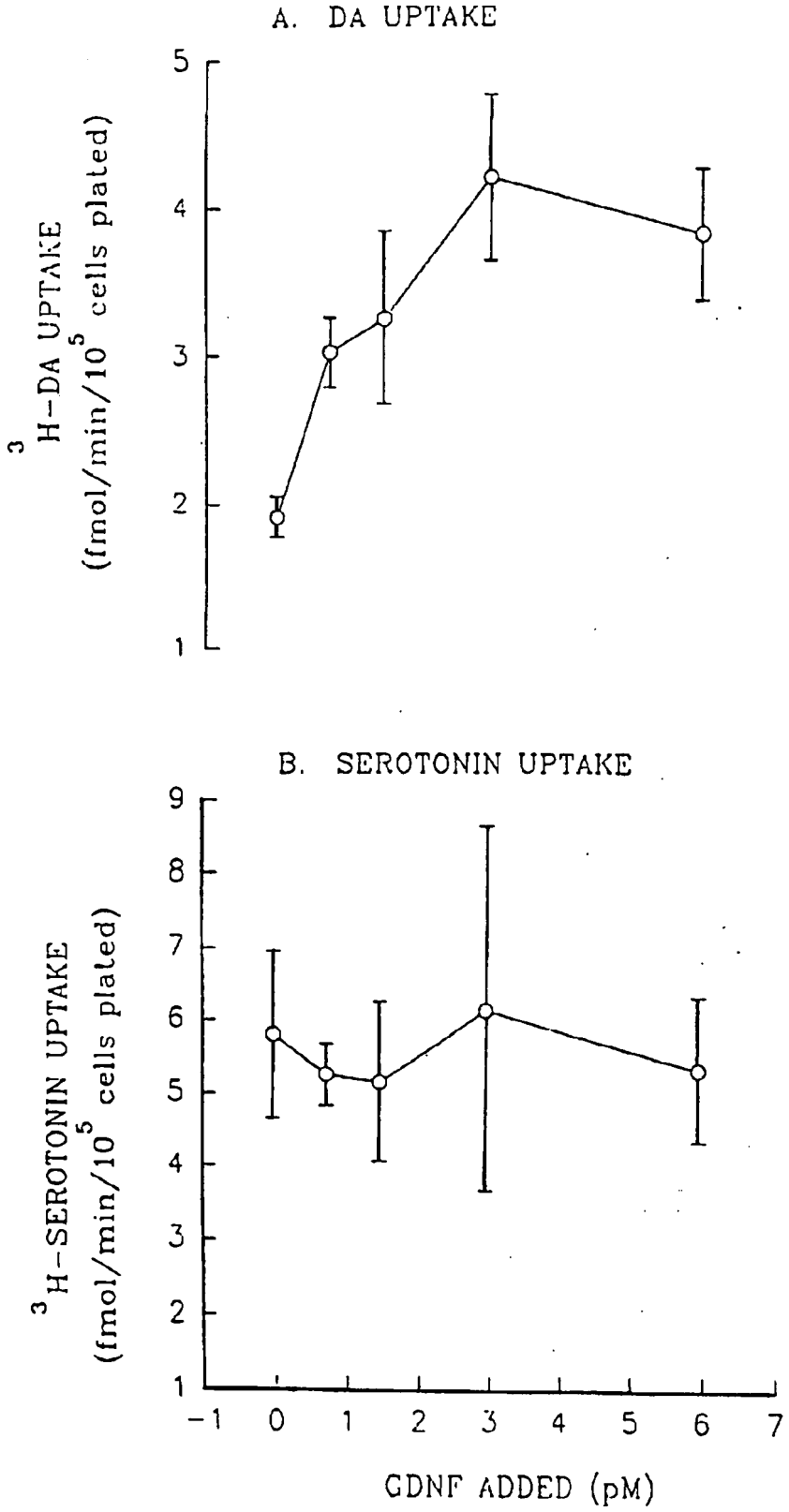
A. DA UPTAKE
^{3}H-DA UPTAKE (fmol/min/10^5 cells plated)
5
4
3
2
1
B. SEROTONIN UPTAKE
^{3}H-SEROTONIN UPTAKE (fmol/min/10^5 cells plated)
9
8
7
6
5
4
3
2
1
−1 0 1 2 3 4 5 6 7
GDNF ADDED (pM)

FIG.24

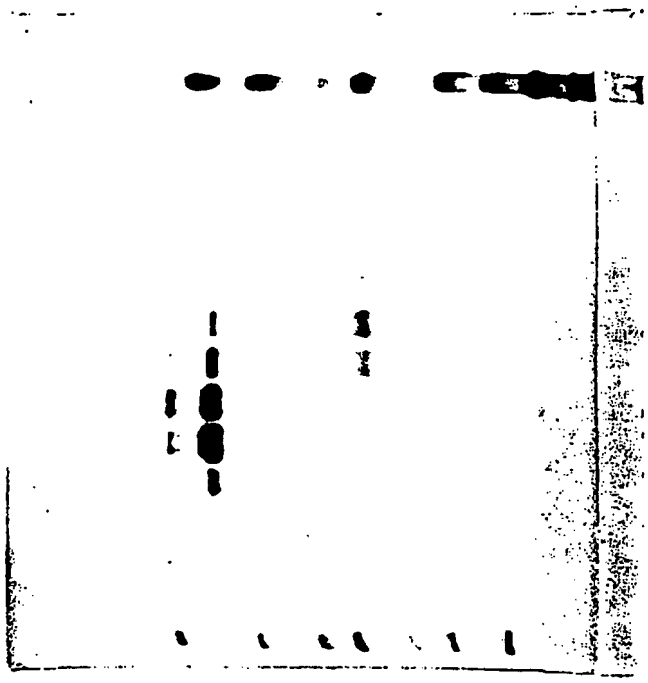

FIG.25

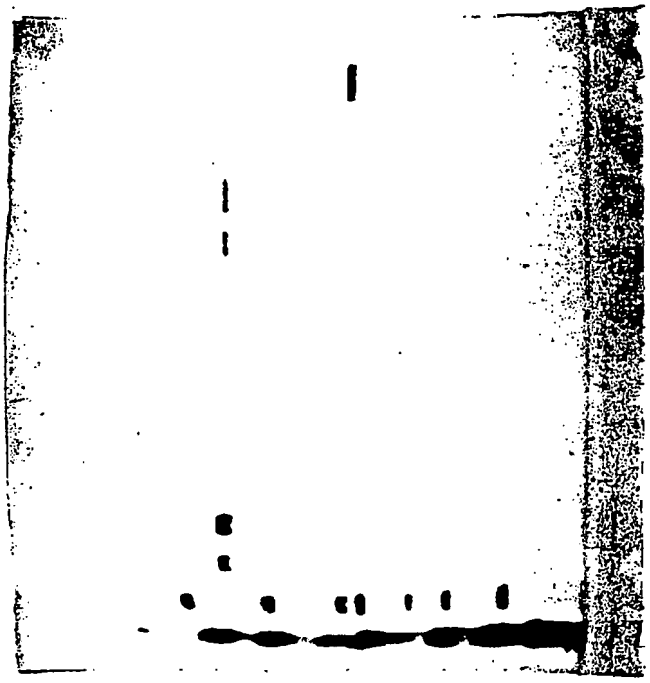

FIG.26

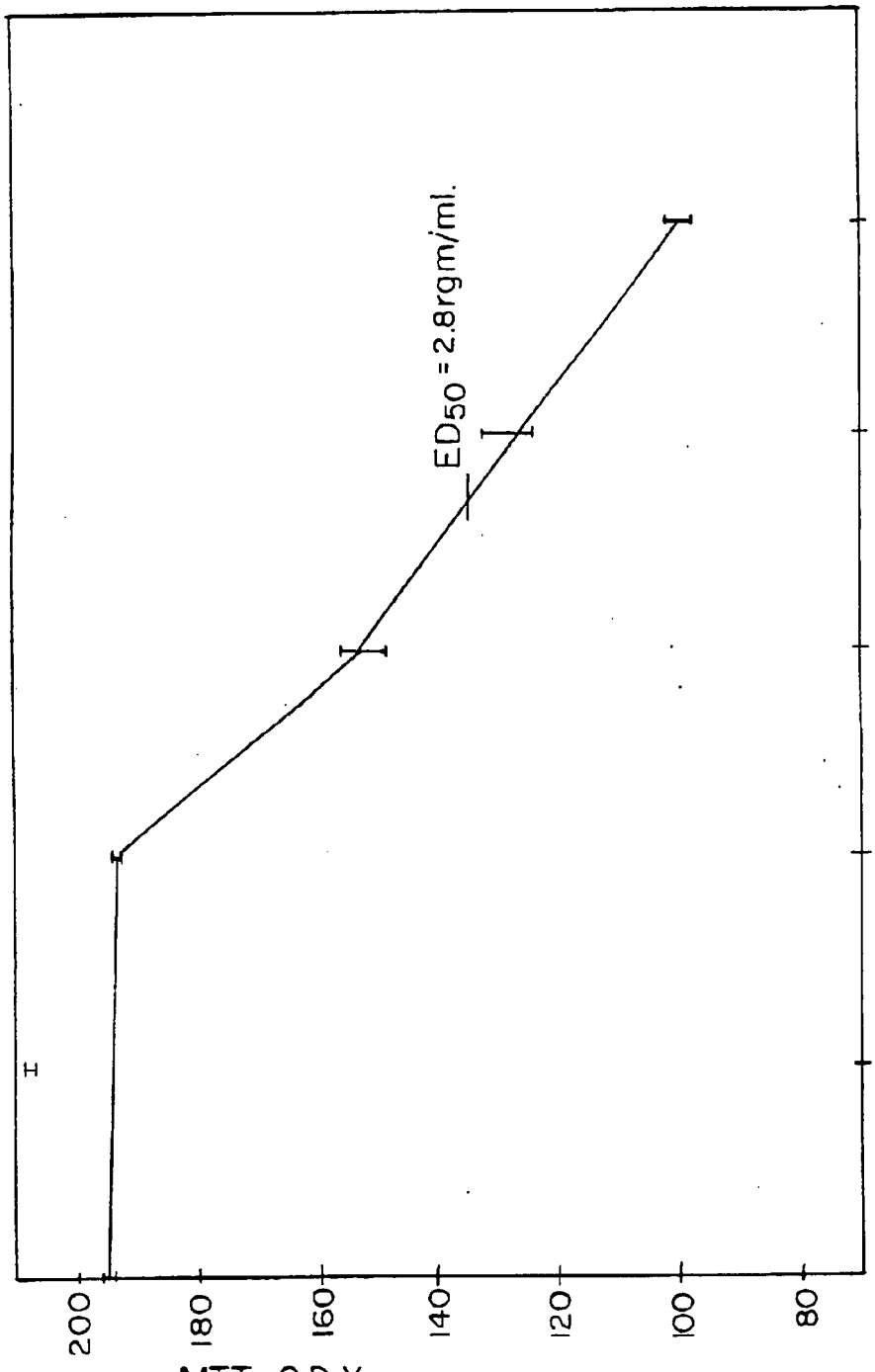
ED50 = 2.8rgm/ml.
0.63
2.0
63
20
63
200
GDNF rgm/ml
200
180
160
140
120
100
80
MTT ODX

FIG.27

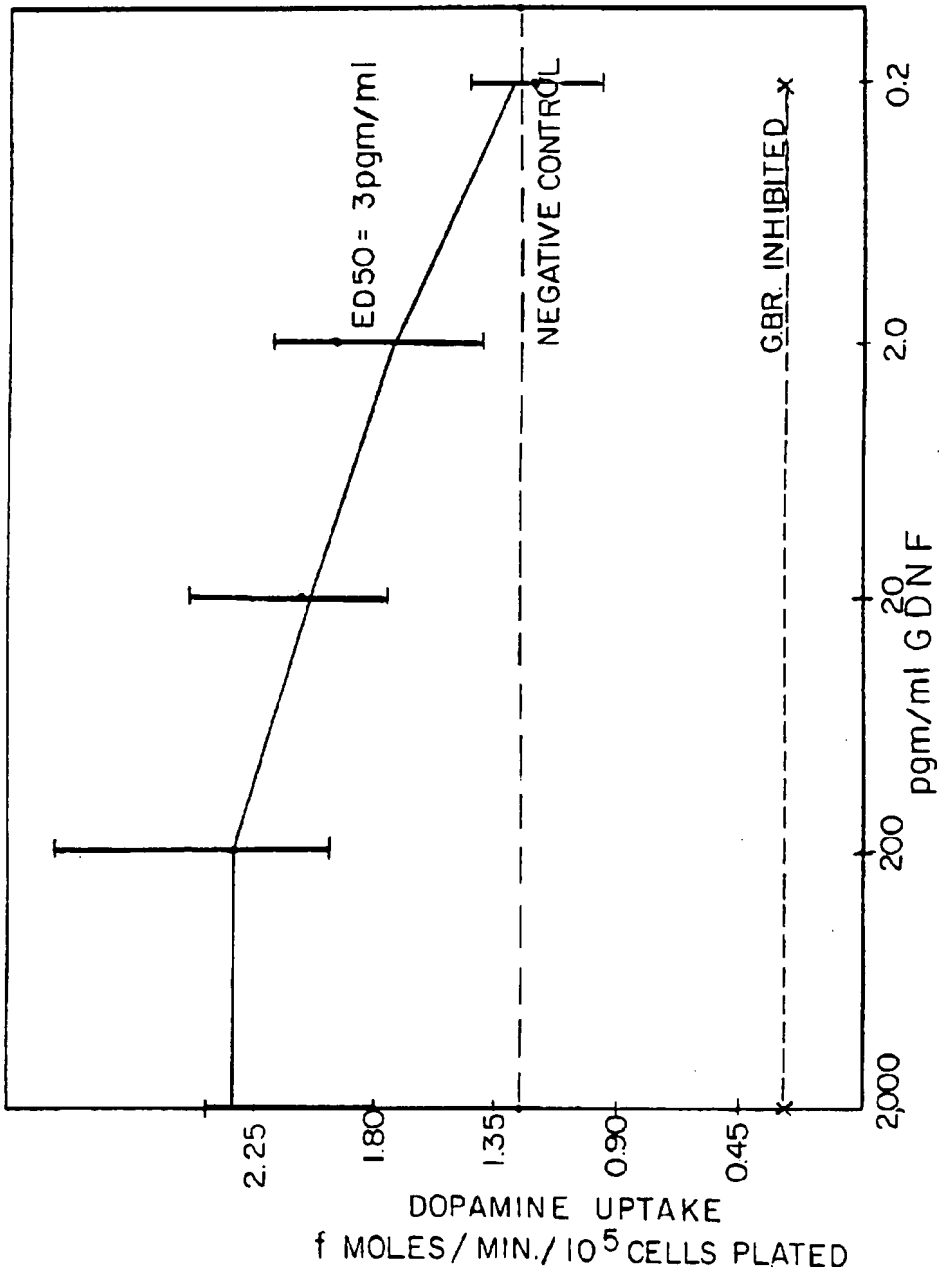
ED50 = 3pgm/ml
NEGATIVE CONTROL
GBR. INHIBITED
0.2
2.0
20
200
2,000
pgm/ml GDNF
2.25
1.80
1.35
0.90
0.45
DOPAMINE UPTAKE
f MOLES / MIN. / 10^5 CELLS PLATED

FIG. 28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07888

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 15/12; C12P 21/06; C12N 1/22; A61K 37/36
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399, 387, 417; 514/12; 536/27; 435/320.1, 252.3, 69.1; 604/890.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, search terms: glial cells, glioblastoma, glial-derived, growth factor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Exp. Cell Res., Volume 120, No. 1, issued 1980. Norrgren et al., "Release of Nerve Growth Factor By Human Glial Cells in Culture", pages 31-40, see abstract.	1-25, 37-40, 56-74
Y	Bio/Techniques, Volume 1, No. 4, issued 1983, Sofer et al., "Designing an OPtimal Chromatographic Purification Scheme for Proteins", pages 198-203, entire document.	1-25
Y	Creighton, "Proteins", published 1984 by Freeman (N.Y.), see pages 39-42	1-25, 37-41, 44-55, 56-59, 60-62
Y	NATURE, Volume 303, issued 30 June 1983, Ullrich et al., "Human beta-Nerve Growth Factor Gene Sequence Highly Homologous to that of Mouse", pages 821-825	26-36, 42-43, 63-69, 44-55, 70-74
Y	BRAIN RESEARCH, Volume 560, issued 1991, Bakhit et al., "Increase in Glia-Derived Nerve Growth Factor Following Destruction of Hippocampal Neurons", page 76-83.	1-25, 37-41, 56-59, 60-62, 70-74, 63-69

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 December 1992	Date of mailing of the international search report 13 JAN 1993
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer SHELLY J. GUEST Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*